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Determination of Sugar in Silages and Forages 1/

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Quantitative analytical methods for sugars in silages and forages have been considerably shortened by use of Waring blendors for extraction (5, 6), ion-exchange resins for clarification (2, 7) and Somogyi's micro copper-reduction reagents for ease of manipulation (3, 4).

Although extracts of many types of fresh green plants may be safely analysed for sugar without clarification (1, 5) Williams and coworkers (7) have shown that extracts of dehydrated plant material must be clarified to remove non-sugar reducing substances present which may be removed by ion-exchange resins according to the technic of Williams et al. (7).

These workers have demonstrated the reliability of the Somogyi reagent for sugar analyses in various types of plant materials and the method is listed in the 6th, 7th and 8th editions of the A.O.A.C. manual. Somogyi's sugar reagent of 1926 (3) is used in the method given in the manual, but his improved reagent of 1945 (4) is used for the determination of starch given in the 8th edition. See also Pucher et al. (8).

The latter reagent has been used in the procedure followed in this laboratory for analysis of sugar in silages and fresh plants. In the revised procedure for sugar analyses of silages and forages, which is reported here in detail, attention may be invited to three points of interest. One concerns the reducing substances which have been encountered in this laboratory in water extracts of ion-exchange resins used for clarification of extracts. The use of resins, therefore, necessitates adequate water washing to minimize blanks, and also the inclusion of resins in blank determinations.

A second point concerns the advantages offered by magnetic stirrings during titrations of the solutions in the 25 mm diameter test tubes used in the Somogyi sugar method. Although magnetic stirring bars, ca. 1 1/4" length stand on end at an angle in test tubes of this diameter, the bars will rotate to give efficient mixing when held over a magnetic stirring apparatus. Under these conditions, titrations are performed rapidly without repeated removal of the tube to mix the contents by shaking. In addition, larger aliquots can be smoothly handled without danger of spillage and poor mixing. Of greater,

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importance, however, is the need to ensure that the CuO precipitate formed in the reduction is stirred sufficiently well to react completely with iodine formed upon addition of KI and H_2SO_4. Experience in this laboratory has shown that 1 minute of rapid stirring by the magnetic apparatus leads to consistent titration values; without this precaution results often gave considerable variation.

As a third point of interest the method, described here, departs from usual procedures in the sequence of dealcoholization and clarification; clarification steps are completed directly on the 80% ethyl alcohol extract. Since the extracts initially contain pigments, ionized substances, and gummy materials, the usual practice of evaporating off alcohol before clarification results in a gummy residue which requires scrubbing to dissolve and often filters slowly. In addition, the sugar is exposed to interaction with these substances at steam bath temperatures during concentration.

These difficulties have been eliminated by the following changes in treatment of the extracts. First, the blendings are filtered through Darco G-60 + Celite 535 mixtures to give practically colorless filtrates. Second, these filtrates, still 80% ethyl alcohol by volume, are deionized directly by mixed resin treatment. These two clarification steps remove ions and extraneous organic matter before the sugar solutions are concentrated on the steam bath to remove alcohol. The sugar solutions, evaporated nearly to dryness, are free of gummy residues, and require no filtration. Further volume enlargement from clarification steps are eliminated. Thus a wider choice of final volume is permitted.

A description of the method follows:

Materials and reagents.
Waring blendor.
Test tubes, 25 mm diameter, 200 mm length.
Boiling water bath and tube rack.
Magnetic stirrer, with teflon-covered stirring bars, 1-1/2" length, 3/8" diameter.
Sintered glass filters, 10 cm diameter, 19 cm depth.

Sugar reagent, Somogyi 1945 (4), prepared with iodate content reduced by one half, Vickery (8).
$Na_2S_2O_3$, ACS grade.
Glucose, certified, Nat. Bur. Standards, add 0.150 gm per liter of water.
Starch solution, 1% freshly prepared.
KI solution, 2.5% from ACS grade KI.
H_2SO_4, 1.8-N and 1.5-N.
Dowex 50, Dow Chem. Co., Midland, Michigan (for activation see company specifications).
Duolite A_4, Chemical Process Company, San Francisco, California (for activation see company specifications).
Ion-exchange resin mixture. 0.5 grams of Dowex 50 plus 1.75 grams of Duolite A_4 (activated resins) are allowed to soak overnight in 75-100 ml

distilled water in a 150 ml beaker and then the water is decanted to minimize the blank.
NaOH, 20%, 2%, ACS grade.
HCl, ACS grade.

Method.

Extraction. Silage or forage materials are chopped by means of a paper cutter into approx. 1/4 inch lengths and are loosened and distributed over a flat surface for sampling. 20-gram samples are comminuted for 10 minutes in a Waring blendor with approximately 400 ml of ethyl alcohol, (80%).

Filtration. The contents of the blendor are poured onto a sintered glass filter containing a filter bed of 60 grams of Darco G-60 + Celite 535 (1+3) sandwiched between two layers of Celite 535, each approximately 1 cm in depth. The combined filtrate and washings (80% ethyl alcohol) are made to 750 ml.

Deionization. An aliquot, 30 ml, is stirred for 15 minutes (magnetic stirrer) with the ion-exchange resin mixture. The deionized solution is decanted and filtered through a glass wool plug inserted in the neck of a powder funnel placed over a 150-ml beaker. The resins are rinsed with 80% ethyl alcohol, which is also decanted onto the filter, and the resins are then stirred for 10 minutes with 25 ml of hot water. The water extract and subsequent rinsing of the resin with approximately 25 ml of hot water are filtered through the glass wool. The filtrate beaker is set on the steam bath; the sugar solution is concentrated to 1-2 ml to remove alcohol.

Inversion. 25 ml of 1-N hydrochloric acid are added to the cooled beaker and the sugar solution allowed to stand overnight to invert.

Neutralization. Sufficient sodium hydroxide (20%) is added to bring the solution nearly to neutrality. Diluted sodium hydroxide (Ca. 2%) is used to complete neutralization to pH 7. The solution is made to volume, 50 ml. Aliquot volumes are usually 5 ml for analysis of extracts from fresh green material and 10 ml in the case of silage.

Copper Reduction. Aliquots are pipetted into 25 mm diameter test tubes specified by Somogyi. An equal volume of sugar reagent is added to the aliquot. The tubes are placed in a rack and immersed in a boiling water bath for 15 minutes. The rack is removed and placed in a pan of cold tap water to cool the solutions.

Standardization. 5 or 10 ml volumes of sugar reagent are mixed with equal volumes of distilled water and also with equal volumes of freshly prepared standard glucose solutions and analyses are made as described under Copper Reduction and Titration. The difference in titer for the tubes containing water and sugar is divided into the mg of sugar present in the aliquot, thereby giving the glucose-equivalent for 1 ml of $Na_2S_2O_3$, "G".

Calculation. A blank is run to include any contribution of reagents or resins to the analysis. From this value, "B", is subtracted the titer, "T", of a silage or forage aliquot. The difference, B-T, is multiplied by "G" to obtain the mg of "glucose" present in the aliquot taken for analysis. Thus, when a 10 ml aliquot has been taken from 50 ml of sugar solution representing 0.8 grams of wet silage material, the sugar may be calculated as follows:

$$\text{Sugar, \% of dry matter} = \frac{(B-T)G \times 100}{10/50 \times .8 \times 10^3 \times DM\ (\%)/100}$$

$$= \frac{(B-T)G \times 62.5}{D.M.\ (\%)}$$

Results. Recovery experiments with standard glucose (National Bureau of Standards) were made to test effect of the Darco G-60 + Celite 535 treatment and also the effect of the resin treatment. In Table 1, satisfactory recoveries are evident.

Table 1. Recovery of Glucose from Darco and Resin Treatments

	Recovery (1.500 mg Glucose Treated)	
	Darco Treatment	Resin Treatment
	1.497	1.495
	1.505	1.502
	1.507	1.499
	1.502	1.500
	1.503	1.494
	1.500	1.492
Average	1.502+	1.497

Satisfactory reproducibility of analyses is shown in Table 2. Comparison of analyses made 19 days apart on silage extracts are listed.

Table 2. Sugar Analyses on Silage Extracts

Sample	Sugar - % Oct. 9	Oct. 28
246	1.85	1.85
248	1.36	1.32
249	1.64	1.69
251	1.47	1.44
267	1.74	1.83
268	2.57	2.54
270	2.11	2.03
272	1.38	1.26
273	1.60	1.60
275	2.12	2.08
278	1.79	1.71
288	2.58	2.55
292	2.20	2.16
295	1.67	1.64
300	1.53	1.54
303	2.71	2.66
304	1.78	1.93
305	2.51	2.45
326	1.97	1.94

Summary.

A method has been presented for the analysis of silage and forage materials. The method differs from conventional procedures in that clarification steps are conducted on 80% ethyl alcohol solutions or extracts of sugar before alcohol is evaporated. Advantages of this change in procedure are given. Blank values for ion-exchange resins and the use of magnetic stirrers for test tube titrations are noted.

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